Urgent symptoms demand immediate exposure of the perforated appendix, after recovery from the shock, and its treatment according to surgical principles.

If delay seems warranted, the resulting abscess, as a rule intraperitoneal, should be incised as soon as it becomes evident. This is usually on the third day after the appearance of the first characteristic symptom of the disease.

BACTERIOLOGY.

By W. Watson Cheyne, M.B., F.R.C.S.,
ASSISTANT SURGEON TO KING'S COLLEGE HOSPITAL, LONDON.

(SECOND PAPER.)

Relations of Bacteria to the Soil on which they Grow.

We have already considered the conditions which favor or hinder the growth of bacteria, and we must now study their mode of nutrition and the changes which take place in the soil in which they are growing. This subject is an extremely complex one owing to the complexity of the chemical substances which are used as food, and the difficulty of ascertaining by analysis the precise changes which have occurred. Hence very little is as yet known about these matters, but there are certain facts which have been made out to which I must shortly allude.

There are two points which have to be considered in this connection, viz.: 1, the assimilation of nutriment by bacteria, the source of their energy, and their respiratory processes; and 2, the products of their growth.

1. As regards the sources of energy; while a small part comes from osmosis, by far the greater part is derived from the processes of intramolecular respiration, the breaking up of complex chemical compounds, probably chiefly of proteids, with the production of CO_2. Intramolecular respiration may go on with or without the presence of free oxygen, but where free oxygen is present additional energy is obtained from the oxidation of these compounds. In some circumstances, where sufficient energy is not obtained by the intramolecular respiration, changes occur in the nutritive media which do not stand in direct connection with the assimilative processes of the plant, viz., fermentative changes. In the absence of free oxygen the necessary energy is obtained, in many cases, from this fermentative process, and hence the meaning of the fact previously mentioned, that if bacteria are cultivated without oxygen they ought to be grown in a suitable fermentescible material.

The substances which exist in the nutritive material do not enter the cells without change and then become broken up; they are first assimilated by the protoplasm and converted into compounds suitable for the further changes. In this process, as well as in the processes of growth, a large part of the energy is used up, for assimilation exceeds destruction; in other words, there are constant growth and new formation of protoplasm.

In the process of assimilation it is necessary for the nutritious substances to be in solution in order that they may enter the cells, and with regard to albumen, many bacteria excrete ferments which in the first instance peptonize the insoluble albumen, and thus render it fit for food. Very little is known with regard to the process of assimilation. With regard to the assimilation of carbon compounds, Flügge[1] says:

> "At present only hypotheses are possible as to what is usually the first carbonaceous assimilation product. In the higher plants, which contain chlorophyll, starch is observed as one of the earliest products; in the lower fungi this is, however, apparently altogether absent, except in a few cases (only in some forms of bacilli and leptothrix). From the different nutritive values of the carbon compounds it may perhaps, according to Nägeli, be concluded that the first assimilation product is composed of three carbon atoms with which hydrogen and oxygen atoms are combined, and which are then united with another similar group of three carbon atoms to form a large molecule of six carbon atoms. The more nearly the nutritive materials approach this hypothetical composition, the less difficulty there is in their assimilation, and the more nutritive they are."

As regards the nitrogenous substances, they are usually increased in complexity of structure as the result of assimilation. This is more especially the case with the ammonium salts, which become combined with carbon. The mineral salts also appear to become combined with complex proteid molecules.

The assimilated materials in part go to form new protoplasm and are in part destroyed by the respiratory process.

2. Many of the products resulting from the vital processes of bacteria are of great importance on account of their relation to life and disease. Some of these products are common to all or a number of different bacteria, others are more or less confined to particular kinds. Flügge gives the following list of the chief products of bacteria:

> "Gases, as CO_2, H, CH_4, H_2S, NH_3; water; sulphur; volatile bodies, such as trimethylamin, alcohol, formic acid, acetic acid, butyric acid; fixed acids, as lactic acid, malic acid, succinic acid, oxalic acid, tartaric acid; sulpho-acids, as taurin, amides of the fatty acids, especially leucin, alanin, etc.; bodies of the aromatic series, as tyrosin, phenol, kresol; reduction products, as indol, hydroparacumaric acid; complex molecules, as carbohydrates, pepton, hydrolytic ferments; finally, coloring matters and poisonous alkaloid substances."

Of these, CO_2, the fatty acids, the amides, and certain aromatic bodies are products common to a number of different bacteria. Carbonic acid is always given off by bacteria in the process of growth, either as a product of the intramolecular respiration or of oxidation and fermentation. Water and a nitrogenous body are also always present as excretory products. Phenol and other aromatic bodies occur chiefly, and perhaps only, in connection with putrefactive processes.

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[1] Fermente und Mikroparasiten.

Among the various results of the growth of bacteria we may refer in detail to three which are of great importance: 1, the production of pigments; 2, the formation of ptomaines; and, 3, the various fermentations.

1. A large number of pigment-forming bacteria are now known, belonging chiefly to the groups of micrococci and bacilli. The color is generally developed only in contact with air; if the bacteria are growing partly in contact with air and partly away from it, the pigment is, in most cases, seen only where the air penetrates. This is easily seen on inoculating a tube of meat jelly with a pigment-forming organism (such as the pink Torula) by plunging an infected needle into the substance of the material. Growth occurs in most instances both at the surface and at the deeper parts of the needle-track; but it will be seen in the case of the torula, for example, that the color is most intense at the surface, and gradually becomes less and less marked the further away the growth is from the air.

The composition of these pigments varies much. Some are soluble in water, and if a fluid is used as a cultivating medium it becomes tinged throughout; others are insoluble in water, and remain in the gelatinous material which unites the bacteria into zoōglœa masses. As has been previously mentioned, the pigment is not usually present in the interior of the cells, but in some instances the cells appear to be tinged by it, as in *beggiatoa rosea-persicina*, in which the pigment (bacterio-purpurin) appears to be in part inside the cell.

As to the nature of these pigments, almost nothing is known. They seem to be allied to the aniline colors.

These bacteria grow and produce their pigment on a great variety of soils. Of these, the most universally suitable are bread paste and the surface of boiled potato. Cohn has shown that an albuminous soil is not necessary for their development, but that certain forms may grow and produce their pigment in artificial fluids (Cohn's solution) containing ammonia and a carbonate, but no trace of albumen.

The various colors are specific products of specific bacteria. The same bacterium always produces the same material under suitable conditions, and, so far as is known, the material is different in the case of each pigment-forming organism. The intensity and precise shade of the color of the pigment depend on several external circumstances. Reference has already been made to the fact that in many instances oxygen is necessary for the development of the color. In other cases the reaction of the medium is of great importance. Thus the pigment of Micrococcus prodigiosus becomes yellowish when an alkali is added, but the red color at once appears when the material becomes acid. This difference of color, according to the reaction of the medium, is well seen in Bacillus cyanogenus (the bacillus of milk), where the blue color is only developed if the milk is acid. It is not, however, necessary, in most cases, that the reaction of the material should always be in the first instance that most suited for the development of the pigment. Thus Micrococcus prodigiosus produces its red pigment on soils which are not themselves acid. This is due to the fact that the micrococci not only produce a pigment, but also an acid. The following are some of the chief pigment-producing bacteria:

Micrococcus prodigiosus[1] is the one which is perhaps best known. It produces a brilliant blood-red color, and frequently appears on bread and other material containing starch, giving rise to the phenomenon of the bleeding host. It also at times grows in milk, which likewise becomes red; an appearance formerly referred to many causes, more especially to a diseased state of the cow, but in reality due to the growth of this organism in the milk after it leaves the body of the animal. The pigment is insoluble in water, and is present either in the glutinous material that unites the bacteria into zoöglœa masses, or in the substratum in which the organisms are growing; the cells themselves are colorless. It is soluble in alcohol and ether. Alkalies turn the color to yellow; acids produce a deep carmine-red tint.

Micrococcus luteus is a very common organism, and frequently appears in the form of yellow drops on the cut surface of boiled potato exposed to the air for a short time. This coloring matter is also insoluble in water, and is apparently contained in the glutinous connecting material.

A number of other micrococci which produce pigments are known, but beyond the fact of this pigment production, very little interest attaches to most of them. Such are *Micrococcus aurantracus* (Schrœter), giving rise to an orange-yellow color, soluble in water. *Micrococcus chlorinus*, producing a greenish-yellow color, soluble in water; acid destroys the color; the material in which it grows becomes strongly alkaline. *Micrococcus cyaneus* (Cohn and Schrœter), causing an intense blue coloration, soluble in water; the color resembles closely that of litmus, and, like it, becomes red with acids, and blue with alkalies. *Micrococcus violaceus* (Schrœter), forming a violet color, also soluble in water. *Micrococcus fulvus*, producing a rusty-red color. In addition to these, are other unnamed micrococci which also produce pigment.

Various sarcinæ give rise to pigments. One, which liquefies gelatine, produces a golden-yellow pigment; another, which does not liquefy gelatine, gives rise to a citron-yellow pigment; and a third forms a delicate pink.

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[1] This organism ought really to be reckoned among the bacilli, but it has been so long known under the name "micrococcus" that it is perhaps better to retain that designation.

Certain pathogenic micrococci also produce pigment. Of these the chief are: *Staphylococcus pyogenes aureus*, giving an orange-yellow color; *Staphylococcus pyogenes citreus*, giving a citron-yellow color; and *Staphylococcus cereus flavus*, giving a yellow color.

Among the bacilli, also, a considerable number produce pigments. Of these the following may be mentioned: *Bacillus prodigiosus*, referred to already under the name *Micrococcus prodigiosus*. *Bacillus indicus*, which was found by Koch in the intestinal contents of a monkey in India; it produces a scarlet color, which becomes blood-red on the addition of ammonia, but regains its scarlet tinge when acetic acid is added. *Bacillus syncyanum*, commonly known as the bacillus of blue milk, which produces a grayish-blue coloring matter. Under the influence of its growth milk becomes of an indigo-blue color. When it grows in pure milk the latter becomes distinctly alkaline, and the color is a slate-gray or pale blue. If, however, the lactic fermentation is going on at the same time, or if acid be added, the intense deep blue color is obtained. For the production of this color, the presence of oxygen or oxidizing agents appear to be necessary. The source of the pigment seems to be, according to Hueppe's researches, the casein, and not the sugar. *Bacillus ruber*, which produces a scarlet color, like that of red sealing-wax. A bacillus is sometimes found in water which produces a beautiful dark violet color (*Bacillus violaceus*) in contact with the air. A dirty red color is also formed by *Bacillus erythrosporus* (Cohn), which is occasionally obtained from the air. *Bacillus pyocyaneus* is the cause of the blue pus occasionally seen in wounds. It produces a greenish-blue color. The pigment can be extracted from the pus by means of chloroform, and in neutral fluids gives a blue color. If the water is acid, the color is red. It thus resembles litmus, and the pigment is called by Fordos *pyocyanin*. Various other bacilli have been obtained from water and air, producing greenish, red, and fluorescing pigments. *Clathrocystis roseo-persicina* (*beggiatoa roseo-persicina*, Zopf; *Bacterium rubescens*, Lankester) produces a peach-colored pigment, investigated by Ray Lankester, and called by him *Bacterio-purpurin*. The pigment is insoluble in water and alcohol, and is apparently contained in the cells. The spectrum of this pigment, as described by Lankester, differs from that produced by the Micrococcus prodigiosus and other pigment bacteria. This organism is found in water in which dead animals are putrefying, in marshes, etc.

Some pathogenic bacilli also produce pigment when growing in various substances, especially on potato. Among these are the *bacillus of glanders*, which gives rise to brownish deposits on potato; *the cholera bacillus*, which, at the temperature of the human body, grows on potato in the form of a grayish-brown layer; *Bacillus alvei*, which produces a yellow color; and the *bacillus of vagus pneumonia* (Jens Schou), the colonies of which on potato have a reddish hue.

Certain other closely allied forms of microörganisms also form pigment, of which may be mentioned various forms of Torulæ: a Torula producing a pink color in contact with the air, a black Torula, and a yellow Torula.

2. Much more important than these pigments are the alkaloid products formed by certain bacteria as the result of their growth in albuminous materials. These have, as yet, been but little investigated on account of the small quantity present and the difficulty of isolating them from the other materials in which they are found. It is only those which occur in putrefying materials which have been isolated, and it has not yet been ascertained what special form of bacterium produces each, or whether the same substance may result from the growth of different kinds of bacteria. It is, however, most probable that, just as in the case of the pigment bacteria, each species of bacteria always gives rise to the same set of products under suitable conditions. It has been long known (since Panum's classical researches) that putrefying materials often contain a chemical poison which, when introduced in sufficient quantity, kills certain animals. Panum found that where a large quantity of putrefying material was injected at one time into animals, death rapidly followed with symptoms of poisoning. To show that this result was independent of the growth of bacteria in the living body, he boiled the fluids for several hours, thus killing all the bacteria; nevertheless, the fluids retained their poisonous qualities, though not to such a degree as before this prolonged boiling. In another experiment he filtered the fluid, boiled it for an hour, evaporated to dryness, digested it with absolute alcohol, and then treated the residue with boiling water, when he found that this watery extract was also poisonous. Since that time various attempts have been made to isolate and analyze these poisonous bodies, but up till quite recently the results have not been satisfactory. Brieger has, however, succeeded in demonstrating the existence of a number of alkaloid substances in decomposing materials and in determining their chemical constitution. As this matter is of great importance, it will be necessary to give some details of Brieger's work.[1]

The name given to these bodies is *cadaveric alkaloids* or *ptomaines* (from πτῶμα, a corpse), and although this term was at first applied only to the bodies formed during putrefaction, more especially during the decomposition of corpses, it is now applied to all the basic alkaloid bodies produced by bacteria. In his early researches Brieger investigated the ptomaines formed during the decomposition of flesh, fish, cheese, and gelatine; in his "further investigations" he deals with the ptomaines found in decomposing human cadavers. These two researches were preliminary to the most important part of these investigations, on which, however, he only touches in these works, viz., the study of the special ptomaines formed by the different species of bacteria, more especially by the pathogenic forms.

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[1] "Uber Ptomaine," von Prof. L. Brieger, 1885, and "Weitere Untersuchungen uber Ptomaine," 1885.

Without going into details as to the methods adopted in isolating the various bodies, the leading processes used in his researches on the cadaveric alkaloids may be briefly indicated. Hydrochloric acid was first carefully added to the materials in order to convert the alkaloids into chlorides, in which form they are not easily destroyed This mixture was then evaporated to a thick syrup and the ptomaine chlorides extracted by means of absolute alcohol. The extract was evaporated and again extracted with absolute alcohol, and this process was repeated several times. In this way those bases which were not readily soluble in alcohol (such as neuridin) were obtained. To the alcoholic solution was then added an excess of an alcoholic solution of bichloride of mercury, and this mixture was allowed to stand for twenty-four hours. The precipitate formed was then boiled in a large quantity of water and filtered. Thus the peptones and albuminates which, after this treatment, were insoluble in water were entirely removed, while the compounds of mercury and ptomaines were soluble in boiling water. On standing, certain of the less soluble bodies (the cholin) crystallized out. By means of sulphuretted hydrogen the mercury was then removed from the solution and the ptomaine chlorides left behind in the fluid; this fluid was evaporated and the residue washed with alcohol, when some alkaloids were dissolved in the alcohol and others were left behind. The dissolved alkaloids were then obtained by the addition of platinic chloride, ptomaine platinochlorides being formed. The processes, however, vary for each base, so that it is not possible to go into details here on these matters.

From meat minced up, mixed with water and allowed to putrefy for five or six days, at the body temperature, several alkaloids can be extracted. The one present in largest amount is called by Brieger *neuridin*, and has the formula $C_5H_{14}N_2$. This body, as will presently be seen, is very constantly present in putrefying materials. It is not poisonous. It can be split into equal parts of dimethylamin and trimethylamin. From the fact that this decomposition shows the close relation of this substance to neurin, the name neuridin was chosen. After removal of the neuridin the remnant is found to be poisonous, and from this the poisonous base *neurin* has been extracted. Another material is also found under the same circumstances, viz., *cholin*. Neurin and cholin have been held by a number of authors to be identical bodies, but Brieger finds that they are different, and holds that neurin ($C_5H_{13}NO$)

is derived from cholin ($C_5H_{15}NO_2$) by the removal of a molecule of water from the latter as the result of the action of the putrefactive bacteria. On animals their effects are different, both of them producing symptoms like those caused by muscarin, but differing much in the dose, ten times more of the cholin chloride being required to kill animals than of the neurin chloride.

In decomposing fish (herring and torsk) neuridin was again found in large quantities, also a poisonous base closely allied to *æthylen-diamin* ($C_2H_4(NH_2)_2H_2O$), but afterward found to differ from it; further, *muscarin* ($C_5H_{15}NO_3$), a base previously found by Schmiedeberg and Koppe, and ascertained by them to be the poisonous agent in a disease of flies caused by fungi (*agaricus muscarius*), and a new non-poisonous alkaloid to which the name of *gadinin* ($C_7H_{17}NO_2$) was given. In putrefying cheese neuridin and trimethylamin were found. From putrefying gelatine were obtained neuridin, muscarin, and dimethylamin. From fresh brain, cholin and neuridin were obtained (but not neurin). Neuridin cannot be got from the other fresh tissues of the body.

Of the various bases found in putrefying materials (neuridin, neurin, muscarin, a body closely allied to æthylen-diamin, gadinin, trimethylamin, triethylamin, and dimethylamin), neuridin is the one which is most constantly present at the commencement of putrefaction. The formation of the others depends to a considerable extent on the materials employed; thus neurin was found only in decomposing meat; muscarin, gadinin, trimethylamin, and the body allied to æthylen-diamin occurred in putrefying fish, and dimethylamin only in putrefying gelatine and putrid yeast.

From decomposing human cadavers the following bodies were isolated: cholin, neuridin, cadaverin ($C_5H_{16}N_2$), putrescin ($C_4H_{12}N_2$), saprin ($C_5H_{16}N_2$), trimethylamin ($(CH_3)_3N$), and mydalein. Of these, cholin and trimethylamin are poisonous in large doses, but mydalein is intensely poisonous, and another base, which was not analyzed, is also noxious. Which of these bodies is present depends greatly on the stage of decomposition. In bodies kept in the cold for twenty-four to forty-eight hours only cholin was present. If the putrefaction had gone on for three days at a warmer temperature, there was less cholin, and neuridin had appeared. After seven days the cholin disappeared entirely, and neuridin and trimethylamin were present. In fourteen days to three weeks new bases were found, and neuridin could no longer be detected. These new bases are, cadaverin, which increases in amount as putrefaction goes on, putrescin, saprin, and the two poisonous bases mentioned before, viz., mydalein and the other unnamed one.

The part of these results which interests us most as medical men is that in which the presence of poisonous alkaloids among the products of bacteria is demonstrated. The poisonous bodies isolated by Brieger were cholin, neurin, muscarin, a body closely allied to æthylen-diamin, trimethylamin, mydalein, and another body of undetermined composition.

Neurin chloride injected into the lymph-sac of a frog, in doses of two to five milligrammes, produces in two to five minutes a paralytic condition; the animal at first reacts on mechanical or chemical stimuli, and reflex action persists to a slight extent for some time. Immediately after the injection the heart beats a little more slowly and more energetically, gradually the relative length of diastole increases, the interval between the beat becomes longer, and arrest occurs in diastole. Atropia often restores the heart's action, and frogs under the influence of atropia generally withstand the action of this toxic substance. Large frogs react slowly, and recover if the dose is one milligramme, but two milligrammes always kill. Of the lower animals, cats are most easily affected. For rabbits the subcutaneous injection of a dose of 0.005 gramme per kilo. of the body weight is necessary to cause the symptoms of poisoning, and a dose of 0.04 gramme per kilo. is required to cause death. Its injection is followed by the appearance of moisture about the nostrils and upper lip, then by profuse salivation, and later by increased secretion from the nose and the lachrymal apparatus. The respirations are quicker and labored, but toward the end they become slower, irregular, and superficial. At first the heart's action is very rapid and strong, but it very soon becomes slow and weak, and the heart stops in diastole. There is often marked contraction of the pupils after subcutaneous injection. This effect is always produced by dropping the solution into the eye. There are increased peristalsis and constant passage of feces; also emission of semen and passage of urine. When fatal doses are employed, there are clonic contractions of the muscles, accompanied by great weakness of the extremities. Atropia is a very active antidote even at a late period, but it does not act as a prophylactic in the case of rabbits. If the poison is administered by the mouth, ten times more is required than by subcutaneous injection.

These symptoms are identical with those produced by muscarin which, likewise, causes in rabbits salivation and flow of tears, contraction of the pupils, profuse diarrhœa, emission of semen, and passage of urine, till finally the animals die with cramps.

Cholin chloride produces symptoms similar to those caused by neurin, but much larger quantities are required, 0.1 gramme per kilogramme of body weight being required to produce the same effect as 5 milligrammes of neurin chloride, and the fatal dose of cholin for rabbits is 0.5 gramme per kilo., or almost ten times the dose of neurin.

The base allied to æthylen-diamin is also poisonous, but is not so violent in its effects as neurin. Even after the injection of large doses into the lymph-sac of a frog there are no effects evident at first; after a time, however, the animal may become lethargic but is easily roused. This is followed by increased respiratory action; the pupils dilate, and the animal dies in some hours without any special symptoms. The heart stops in diastole. In mice and guinea-pigs the effects are more marked. Within a short time after the injection of small doses there is profuse secretion from the nose, mouth, and eyes; dilatation of the pupil and exophthalmos. Then there is violent dyspnœa, which often lasts till the death of the animal several hours (sometimes twenty-four) after the injection. Small doses had no immediate marked effect on rabbits beyond a transient and slight salivation and increased frequency of respiration, but several of these animals were found dead after twelve to twenty-four hours.

The action of mydalein, which is also apparently a diamin, is very characteristic. Small doses injected into rabbits and guinea-pigs produce, very quickly, increased secretion from nose, mouth, and eyes, dilated pupils, injection of the vessels of the ear, rise of temperature of 1° or 2° C., and at times shivering. By and by, these symptoms pass off and the animal recovers. If large doses (about one-half of a centigramme) are injected into a guinea-pig, the effect is very violent and always ends in the death of the animal. The secretion from the organs provided with smooth muscular fibres is extremely profuse, the saliva and feces mingle so that the animal always lies in a wet mess, more especially as locomotion is interfered with. Exophthalmos occurs, the dilated pupils are difficult to examine on account of the secretion from the lachrymal glands (this secretion is white and turbid). When the action of the poison has reached its height the animal falls down, because first the hind and then the front extremities become paralyzed; there are fibrillary twitchings in the different groups of muscles; respiration becomes more and more labored and panting, at times the animal leaps up gasping for air, raises its head and then again sinks down and lies in its feces, making slight movements with its limbs. The temperature gradually falls, the movements become weaker and weaker, and finally the animal dies. On post-mortem examination the heart is found to have been arrested in diastole, the intestine and bladder are contracted, but otherwise there is nothing abnormal.

The other poisonous substance obtained from putrefying human cadavers caused increased intestinal peristalsis in guinea-pigs and rabbits which lasted several days, and, on account of the continued evacuations, led to great weakness of the animal. Beyond this, no abnormal effect was noticed.

Brieger mentions a few preliminary attempts which he has made to search for ptomaines among the products of pure cultivations of the pathogenic bacteria. These have not yet led to any definite result. Friedländer's pneumococcus breaks up carbohydrates into formic acid, acetic acid, and ethyl-alcohol; Brieger's bacillus, which was obtained from human feces and which kills guinea-pigs, gives propionic acid as the chief product of its growth in grape sugar.

The typhoid bacilli give considerable quantities of ethyl-alcohol and small quantities of volatile fatty acids, more especially acetic acid. They break up grape sugar into lactic acid. Brieger was also able to obtain from their cultivations in meat infusion a new ptomaine, small quantities of which produced marked effects on guinea-pigs. There were slight salivation and increased frequency of respiration. The animals lost control over the muscles of the extremities and back, without, however, the occurrence of any true paralysis; the pupils become dilated and ceased to react to light. There was also profuse diarrhœa, and the animals died in twenty-four to forty-eight hours. On post-mortem examination the heart was found strongly contracted, the lungs hyperæmic, the remaining internal organs pale, the intestine firmly contracted and its walls pale. This substance was probably a ptomaine, but its exact composition was not definitely made out. From cultivations of Staphylococcus pyogenes aureus a new ptomaine was also obtained, which, however, was apparently not poisonous.

Not the least important of the results of the growth of microorganisms are the ferments and the products of fermentation.

A number of microörganisms produce small quantities of substances which act as ferments in the same way as ptyalin, pepsin, trypsin, etc., act. This substance, the so-called chemical ferment, gives rise to fermentative changes, a very small quantity of the ferment being capable of causing alteration in a large quantity of material. These ferments are necessary agents in the nutrition of many microörganisms, converting substances which are unsuitable for food into easily assimilable materials. Of these we must note more especially the diastatic and the peptonizing ferments. Diastase converts starch into glucose in faintly acid solutions. It is excreted more especially by the *Bacillus butyricus.* Invertin is produced constantly by the ordinary *Torula cerevisiæ,* and converts cane sugar into glucose (dextrose and lævulose). The common yeast plant cannot assimilate cane sugar, but nevertheless it grows readily in solutions containing it, this ferment being produced and rapidly converting the cane sugar into glucose, which is then easily acted on. Certain bacteria (*e. g.*, *Bacillus amylobacter*) produce ferments which convert cellulose into glucose, and others which convert milk sugar into the same substance. The peptonizing ferment converts albuminous substances into peptones and is produced by various bacteria, more especially by the putrefactive organisms. There are other ferments less frequently found, some of which act on fat, converting it into fatty acids and glycerine, others on amides, etc. In the case of the fermentation of urea a chemical ferment has been isolated as a product of the micrococci which are apparently the causes of the ammoniacal fermentation of urine. These bodies appear to have a hydrolytic action; they enable the molecule acted on to take up one or more molecules of water and then cause it to split up into two or more complex molecules without undergoing any alteration themselves. There is, however, a limit to the amount of material on which they can act, diastase, for example, being able at most to convert 2000 times its weight of starch into glucose. Flügge gives the following hypothetical formula of the action of these bodies:

$$\text{Diastase:}\quad \underset{\text{(Starch.)}}{C_{24}H_{40}O_{20}} + 3H_2O = \underset{\text{(Dextrin.)}}{C_6H_{10}O_5} + \underset{\text{(Dextrose.)}}{3C_6H_{12}O_6}.$$

$$\text{Invertin:}\quad \underset{\text{(Cane sugar.)}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{(Dextrose.)}}{C_6H_{12}O_6} + \underset{\text{(Lævulose.)}}{C_6H_{12}O_6}.$$

$$\text{Urea ferment:}\quad \underset{\text{(Urea.)}}{CO(NH_2)_2} + 2H_2O = \underset{\text{(Ammonium carbonate.)}}{CO_3(NH_4)_2}.$$

Differing from these chemical fermentations in many ways are the fermentations caused by many forms of bacteria. They result in much more extensive and continuous changes in the fermentescible material, these changes being, as a rule, only brought about by one special form of microörganism. In some cases, however, various different organisms set up a similar process, though there is generally only one form which acts to a very marked extent. How these changes are brought about is not known, and probably the process differs in different instances. In any case, there is no doubt that the changes are due to the growth of living organisms, and not, independently of the organisms, to oxidization, molecular change, etc., as was formerly supposed. The evidence in favor of this statement is so strong and so well known that it is unnecessary to discuss it.

Various views have been propounded at different times to explain the action of the microörganisms. It might, for example, be supposed that these plants act by producing hydrolytic ferments which split up the fermentescible material in the manner before described. The only instance, however, in which the existence of such a substance has been demonstrated is in the case of the ammoniacal fermentation of urine. This has been shown to be due to the growth of an organism in the urine —the *Micrococcus ureæ* (Cohn)—and not to the vesical mucus, as was formerly supposed. Musculus demonstrated that by adding absolute alcohol to ammoniacal urine, a precipitate was obtained which could be filtered and dried. This precipitate transformed urea into carbonate of ammonia.[1] These results have been confirmed by Lea, of Cambridge. According to Pasteur, the cause of the fermentation is not the production of a ferment, but the breaking up of the chemical compounds by the growing plants in their search for nutriment, more especially for oxygen. Again, one might suppose that the organisms while living in various substances feed on them, and the products of the fermentation may be either the portion of the food which has been rejected by them, or products formed in and excreted by the organisms. There is, however, a considerable amount of evidence that the changes often occur outside the cells altogether. Nägeli has pointed this out with reference to the frequent formation of acetic ether in connection with alcoholic fermentation. Acetic ether can only be formed when acetic acid and alcohol are both in the nascent state. As these bodies do not result from the growth of the same organism, but of different organisms, it follows that they combine outside the cells, and that therefore the acetic acid and alcohol are nascent—in other words, are being formed outside the cells. Nägeli's theory is that "fermentation takes place in this way, that as the result of the intramolecular activity in the protoplasm intense movements are set up, and that outside the cells there are chemical molecules which are set into active motion by these movements, so that breaking up of these molecules results."[1] In all probability fermentation occurs in different ways in different cases. In any case, the fermentative action is a process distinct from the intramolecular respiration, and goes on alongside of it. Hence in the resulting material there are found not only the products of the fermentation but also the products of the life of the plant, though the former are present in enormously larger quantities than the latter.

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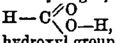
[1] Bacteria, Magnin.

As to the chemical changes which occur in fermentation, Hoppe-Seyler (see Flügge) makes the following suggestion:

> "In all cases carbonic acid is produced; for this, new combinations between carbon and oxygen are necessary, and these are rendered possible by the breaking up of the existing union between oxygen and hydrogen, carbon and hydrogen, and carbon and carbon. In the fermentation of formic acid,  $\mathrm{H{-}C}\begin{smallmatrix} /\!\!/\mathrm{O} \\ \diagdown\mathrm{O{-}H} \end{smallmatrix}$, the union between the oxygen and hydrogen atoms in the hydroxyl group, and also the union between the hydrogen and carbon atoms is broken, the free hand of the oxygen atom attaches itself to that of the carbon atom, the hydrogen atoms unite, and thus we have H_2 and CO_2. In a similar manner in all fermentations there is a wandering of the oxygen atom from hydrogen to carbon, the point of attachment of the oxygen atom to the carbon one being obtained by the dissolution of the union between carbon and hydrogen and carbon and carbon. In short, the group carboxyl is formed, while, on the other hand, reducing compounds of hydrogen with hydrogen or carbon with hydrogen appear. . . . Nevertheless, the wandering of the oxygen atom which leads to the breaking up of the molecule only occurs when the molecule is not too large in relation to the number of displaceable carbon atoms. If there are numerous carbon atoms in union with one another while only one OH group is present, as in many benzol derivatives, in the higher fatty acids, etc., which can go to form carboxyl, then no such change occurs in the molecule; on the other hand, this change is possible when there are several oxygen atoms which can form new carbon compounds, as in the fer-

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[1] Quoted from Flügge, op. cit.

mentation of glucose, where three carboxyl groups are present in each molecule. In the case of acetic acid, fermentation only occurs with difficulty for this reason, and in propionic acid it is still more difficult, for here, as in formic acid, there is only one oxygen atom free to form a carboxyl group, while the molecule is decidedly larger than that of formic acid; on the other hand, fermentation occurs much more easily in the case of the oxyacids (glycocholic acid, lactic acid, etc.), because in these there is a second hydroxyl group, and thus a second displaceable oxygen atom. Consequently, the following are not fermentescible substances: carbonic acid, amines which contain no oxygen; further, the large molecules of the higher fatty acids and the benzol derivatives which are not rich in oxygen. On the other hand, the following must be fermentescible: the higher alcohols, the lower fatty acids down to propionic acid, the oxyacids, and the multibasic acids of the fatty series, the carbohydrates, and the proteids."

The first fermentative change which was demonstrated to be due to the growth of living organisms is the alcoholic fermentation of sugar. I have previously referred to experiments which showed that the unboiled juice of the grape could be preserved without undergoing fermentation if taken from the grape and kept with certain precautions. Pasteur's experiments led him to the following conclusions:

"The boiled juice of the grape never ferments when kept in contact with air which has been deprived of the germs suspended in it.

"Boiled grape juice ferments when a very small quantity of water is introduced, in which the surface of the grape and the branches of the vine have been washed.

"Grape juice does not ferment after the introduction of this water if the latter has been previously boiled and allowed to cool.

"Grape juice does not ferment when a small quantity of the juice taken from the interior of a living grape is added to it."

On the other hand, grape juice does ferment if certain microörganisms be introduced, the sugar being converted into alcohol, carbonic acid, and other products. The special organism which produces this change is the yeast plant, or Torula cerevisiæ. Other forms of torulæ also set up the change, though to a much less extent, as well as some of the lower fungi, such as the Mucor racemosus, when free oxygen is absent. The latter is, however, not a true fermentation, but rather the result of the intramolecular respiration of the plants. Cane sugar cannot undergo this fermentation; it must first be converted into dextrose and lævulose. This is done by means of the invertin formed by the torulæ.

The chemical change which occurs is much more complex than was at first supposed, because not only are alcohol and carbonic acid produced, but also small quantities of glycerine, succinic acid, and often traces of acetic acid, amyl-alcohol, etc. These by-products apparently also owe their origin to the torulæ, and show that the fermentative change is probably a very complex one. The fermenting fluid must not contain too much sugar, otherwise the torulæ cannot grow; thirty-five per cent. of sugar is said to be the highest limit. The growth and fermentation also cease when the amount of alcohol in the fluid has reached 12 to 14 per cent. Of the sugar broken up, 95 per cent. appears as alcohol and carbonic acid, 2½ to 3½ per cent. appears as glycerine, and 0.4 to 0.7 per cent. as succinic acid.

The lactic acid fermentation is due to a small bacillus, the *Bacterium lactis* (Lister). These bacteria are very short, thick cells, about half as long again as they are broad; when unstained they seem to be oval bodies. They are generally arranged in pairs, sometimes they are in chains. They are constantly found along with a great variety of other microörganisms in milk got from a dairy, and they increase in number as the milk becomes sour. They have been separated from the other bacteria and cultivated pure in various infusions, in nutrient jelly, etc., and their introduction into sterilized milk is followed by souring and coagulation of the milk due to the lactic fermentation. Though this is the chief organism which causes lactic fermentation, a number of other organisms, such as the typhoid bacillus, produce small quantities of lactic acid when grown in solutions containing grape sugar. The *Bacterium lactis* can cause fermentation in solutions of cane sugar, and therefore it is supposed that it forms a ferment which transforms the cane sugar into glucose, but this ferment has not yet been isolated. The precise changes that occur in the glucose have not been made out. A very large proportion is converted into lactic acid, but beside lactic acid there is always the development of carbonic acid gas, and there are also other by-products which have not yet been thoroughly investigated. When a certain and not very large quantity of lactic acid has been produced, the growth of the organisms, and consequently the fermentation, ceases. But if chalk be added to the milk, the lactic acid is neutralized, lactate of lime being formed, and much larger quantities of lactic acid are obtained.

The butyric acid fermentation is also due to a bacillus which is longer and larger than the Bacterium lactis. Pasteur thought that these bacilli could only cause the butyric fermentation in the absence of free oxygen; indeed, he went further and thought that oxygen killed them. This is, however, not the opinion of more recent investigators, more especially of Hueppe. Hueppe finds that these organisms can grow in milk and set up fermentation under conditions which have nothing to do with the presence or absence of free oxygen, and that the conditions in relation to air under which they act in producing fermentation are precisely the same as those of other bacteria. If sterilized milk be inoculated with a pure cultivation of this bacillus and kept at the body temperature, in two days a clear, somewhat yellowish fluid may be seen under the layer of cream. This fluid increases in amount as time goes on. At the bottom of the vessel is the coagulated casein, but after a time this coagulum becomes dissolved and peptonized, and then further broken up by the bacilli. According to Fitz, after ten days fermentation in an artificial cultivating fluid containing 100 grammes of starch there are formed 34.7 grammes of butyric acid, 5.1 grammes of acetic acid, 0.33 gramme of succinic acid, and 1 gramme of ethylic alcohol.

The facts as to the viscous fermentation of sugar are not so well known, but, according to Pasteur, the microörganism at work is a micrococcus. The fermenting fluids become viscous, so that they can be drawn out in long threads. The change which occurs is that the sugar becomes transformed into a form of gum, mannite, and carbonic acid. According to Flügge, 100 parts of sugar give 51.1 parts of mannite, 45.5 parts of gum, and 6.2 parts of carbonic acid, and he explains this change by the following formula:

$$\underset{\text{(Dextrose.)}}{(50C_6H_{12}O_6)} = 12\underset{\text{(Gum.)}}{(C_{12}H_{20}O_{10})} + 24\underset{\text{(Mannite)}}{(C_6H_{14}O_6)} + 12CO_2 + 12H_2O.$$

The alkaline fermentation in urine has already been referred to in connection with the subject of chemical fermentations. It is apparently due to a micrococcus (*Micrococcus ureæ*), and the breaking up of the urea into carbonate of ammonia and water seems to be the result of the action of a chemical ferment formed by these organisms. That this change is really dependent on microörganisms, and not on the bladder mucus, is shown by the experiments in which normal urine is taken from the bladder with precautions against contamination, this urine, though unboiled, remaining for an indefinite time unaltered. Among the most striking experiments in this direction are those of Cazeneuve and Livon,[1] of which the following is an example: Remove the urinary bladder from the body of an animal, dip it for a few seconds into paraffin at 100° C., so as to destroy any living organisms which may have fallen on its wall, then plunge it into paraffin at 45° C., so as to get a thicker coat, and then hang it up. Even after three days it will be found that the fluid which has exuded from the bladder into the paraffin cup is clear, acid, and devoid of organisms. Remove the cup and expose the bladder to the air for a few hours, then give it a new coating of paraffin at 45° C. (which will not kill the germs which have fallen on the bladder during the exposure), and leave it again for three days. The fluid outside the bladder will then be found to be ammoniacal and to contain organisms. Similar results were obtained when the urine had been previously rendered alkaline by the administration of soda or potash.

The acetic fermentation is the conversion of alcohol into acetic acid, and is brought about chiefly by a small bacillus which grows on the surface of the fluid and forms dense, compact zoöglœa masses. This organism develops best in fluids containing a small quantity (one or two per cent.) of acetic acid, and there must not be too much alcohol present (at most ten per cent.).

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[1] Revue Mensuelle, 1877, p. 733.

The putrefactive fermentation is a very complex change, and one about which very little is known, except that it is caused by the growth of microörganisms in the putrefying material. Probably there are a number of different fermentative processes occurring singly or together, which are accompanied by the development of a putrid smell. Nor has much been made out as to the microörganisms concerned in the process. Pasteur's researches led him to the view that, when a substance putrefies, two classes of microscopic organisms are at work—the first, in point of time, abstracting the free oxygen from the material; and the second, which then appear, being unable to live in free oxygen, but nevertheless requiring oxygen for their growth, obtaining it from the chemical compounds present. The result of this extraction of oxygen is the breaking up of these compounds and the rearrangement of their elements to form new compounds which constitute the products of putrefaction. Later observations have thrown doubt on the accuracy of this theory, and the probability seems to be that the organisms which produce putrefaction act as well in the presence of air as do the other fermentative organisms. Rosenbach[1] has found three different organisms which produce a putrid odor, and one of these is, he thinks, the common agent in this change. This organism, which he calls *Bacillus saprogenes, No. 1*, was obtained in the first instance from the air of a hospital ward, and produces an intensely putrid odor. It is a comparatively large bacillus, often containing a spore at one end, and it grows readily in a number of media. If inoculated on a mixture of water and egg albumen, free access of air being permitted, it breaks up the albumen rapidly, the fluid becomes greenish-yellow, and when the flask is opened a horribly offensive odor is perceived. If cultivated without oxygen, it causes hardly any putrefactive change in the material. A second bacillus which Rosenbach obtained produces a somewhat different smell, and acts somewhat more vigorously in the absence of oxygen than does No. 1. Although there may be only one or two organisms which produce the foul smell of putrefying material, there is no doubt that a large number of different species of organisms take part in the whole process of the destruction of albuminous materials. Probably, also, the presence of plenty of oxygen leads to the rapid destruction of the foul-smelling products, and to the more rapid and complete oxidation of the material. Flügge suggests the following as the course of events in the putrefactive process: When bacteria gain access to a putrescible medium, they at first increase in number, often with the formation of peptonizing ferments. The soluble albumen formed by means of these ferments then undergoes decomposition, which most probably occurs in this way, viz., that amido-derivatives of the fatty series (especially amido-acids), nitrogenous bodies of the aromatic series, sulpho-acids (taurin), and perhaps peptone-like bodies are formed. Probably the products which are first formed are rapidly broken up—*e. g.*, the amido-acids into ammonia and fatty acids, the latter being still further broken up by liberation of CO_2 into H and CH_4. Thus, for leucin the following fermentative change has been ascertained:

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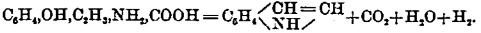
[1] Microörganismen bei den Wundinfectionskrankheiten des Menschen.

$$C_5H_{10},NH_2,COOH+2H_2O=C_4H_9,COOH+NH_3+CO_2+2H_2.$$

(Leucin.) (Valerianic acid.)

In the case of tyrosin, also, one must suppose that it very quickly undergoes decomposition, as it is only found in considerable quantities at the commencement of putrefaction. According to Nencki, the following may be the change:



$$C_6H_4,OH,C_2H_3,NH_2,COOH=C_6H_4\genfrac{<}{>}{0pt}{}{CH=CH}{NH}+CO_2+H_2O+H_2.$$

(Tyrosin.) (Indol.)

According to Baumann, tyrosin, when putrefying in the presence of oxygen, breaks up into hydroparacumaric acid, paroxyphenyl acetate, paracresol, phenol.

In the superficial layers of the earth, the organic material undergoes a process of decay which leads to the so-called "nitrification," nitrites and nitrates, sulphates and carbonic acid being formed. For this process a porous condition of the soil freely admitting air is necessary; if the soil is compact, putrefactive processes occur. In soil, also, from the decomposition of plants, humin appears, and, in marshes, CH_4.

(To be concluded.)

THE TENDON-JERK AND MUSCLE-JERK IN DISEASE, AND ESPECIALLY IN POSTERIOR SCLEROSIS.[1]

By S. Weir Mitchell, M.D.,
MEMBER OF THE NATIONAL ACADEMY OF SCIENCES,
AND
Morris J. Lewis, M.D.,
OF PHILADELPHIA.

Our recent researches[2] have, as we believe, given to symptomatology an enlargement as to certain nervous maladies. The present brief paper is an effort to apply what we have learned to a single disease—posterior sclerosis of the cord. This disease was chosen because we were able to gather readily for examination a representative group of cases, and also because its natural history descriptions, having come from masters of their art, are both definite and reasonably complete.

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[1] Read before the Association of American Physicians, June 17, 1886.

[2] Physiological Studies of the Knee-jerk and of the Reactions of Muscles under Mechanical and other Excitants, Philadelphia Medical News, February 13 and 20, 1886.